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Genotypic Differences in Mineral and Nitrogen Homeostasis under Water Stress in Chickpea (*Cicer arietinum* L.): Crucial Role of Nitrogen Source and Symbiotic Efficiency

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This study compares the water-stress responses of two Moroccan chickpea varieties (*Cicer arietinum* L.), Rizki (tolerant) and Bouchra (sensitive), under two nitrogen sources: mineral and symbiotic (via the *Rhizobium* strain B95). The experiment was conducted under controlled conditions on an inert substrate, applying six levels of drought stress ranging from 100% to 50% of field capacity. The results showed that biological nitrogen fixation (BNF) was markedly inhibited at 60% of field capacity in both varieties, highlighting the sensitivity of rhizobial symbiosis to water deficit. Rizki maintained better growth, greater stability of photosynthetic pigments (chlorophylls and carotenoids), more resilient nodular activity, and higher accumulation of osmoprotectants (proline and soluble sugars). In contrast, Bouchra exhibited a pronounced reduction in biomass and a rapid degradation of pigments and nodulation. A specific accumulation of manganese (Mn^{2+}) in the stems, observed only in Rizki under stress, suggests an ionic adaptation mechanism linked to drought tolerance. These findings highlight two promising markers for varietal selection: (i) carotenoid stability as an indicator of photosynthetic protection, and (ii) Mn^{2+} accumulation in stems as an ionic adaptation trait.

Keywords: *Cicer arietinum*, water stress, biological nitrogen fixation, varietal tolerance, manganese, carotenoids.

INTRODUCTION

Chickpeas (*Cicer arietinum* L.) are among the most widely grown legumes in semi-arid regions, where the availability of water and mineral nitrogen represents major limiting factors for agricultural productivity (Saxena et al., 2013; Plett et al., 2020). In these environments, biological nitrogen fixation (BNF), through symbiosis with rhizobia, constitutes a sustainable alternative to reduce dependence on nitrogen fertilisers (Graham & Vance, 2003). However, this symbiosis is particularly vulnerable to drought stress, which affects both the host legume and its microsymbionts (Ladrera et al., 2007). Water stress disrupts several processes essential for nodulation and BNF. It reduces nodule formation and activity, limits the supply of photosynthates, and compromises ionic homeostasis (Purcell et al., 1997; Serraj et al., 1999). Nodules, which are highly specialised structures, require a delicate balance of oxygen, water, and ions to maintain nitrogenase activity (Hernández-Jiménez et al., 2002). Water deficit alters membrane permeability, promotes the accumulation of toxic ions (Na^+, Cl^-), and decreases the concentrations of essential cations such as K^+ and Ca^{2+}, directly affecting enzymatic activities (Salmerón et al., 1991; Chammakhi et al., 2022; Khan et al., 2024). Drought-induced ionic imbalances disrupt haemoglobin synthesis, pH regulation, and energy production, processes crucial for effective symbiosis. (Tejera et al., 2005; Li et al., 2018).

Water stress profoundly affects mineral nitrogen nutrition (nitrate and ammonium) in legumes, altering both the availability of these forms in the soil and their absorption,

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transport, and assimilation by the plant. The reduction in root water conductivity under drought stress conditions limits the mass flow of ions to the roots, thereby decreasing NO_3^- and NH_4^+ supply, particularly in soils where nitrate mobility depends on water availability (Gruber et al., 2013).

Water deficit reduces the expression and/or activity of root transporters involved in mineral nitrogen, thereby compromising root absorption (Wang & Wu, 2013). In addition, the intracellular assimilation of mineral nitrogen is severely disrupted: the activity of nitrate reductase, a key enzyme in the reduction of nitrate to nitrite, is inhibited by both the decrease in leaf water potential and the accumulation of reactive oxygen species (ROS) under stress (Foyer et al., 2006).

Similarly, the activity of glutamine synthetase (GS), responsible for ammonium assimilation, decreases, limiting amino acid synthesis and affecting the remobilisation of nitrogen toward growing organs.

For chickpea (*Cicer arietinum* L.), a species mainly cultivated in semi-arid areas, several studies have reported a significant decrease in mineral nitrogen uptake under drought stress, particularly during the sensitive stages of flowering and pod filling (Khan et al., 2010; Garg & Singla, 2014). This reduction is accompanied by a reduction in nitrate reductase activity and a partial accumulation of unassimilated nitrate in the tissues, indicating an imbalance between nitrogen uptake and metabolism.

Furthermore, chickpea exhibits a preference for NH_4^+ uptake under stressful conditions, probably due to the lower energetic cost compared with NO_3^- assimilation, although this strategy remains limited by the naturally low availability of NH_4^+ in most cultivated soils (Vadez et al., 2012b). Thus, water stress compromises not only the acquisition but also the efficient use of mineral nitrogen in chickpea, leading to direct consequences on its growth, yield, and protein quality.

Despite this knowledge, few studies have compared genotypic responses to water deficit according to nitrogen source (mineral vs. symbiotic). In particular, the physiological and ionic mechanisms underlying varietal tolerance in chickpea remain poorly understood. Therefore, identifying discriminating traits, such as the stability of photosynthetic pigments or specific ionic markers, could provide valuable criteria for varietal selection.

This study compares two contrasting Moroccan varieties, Rizki (tolerant) and Bouchra (sensitive), cultivated either with mineral nitrogen or in symbiosis with the *Rhizobium* strain B95, under different levels of drought stress. We evaluated their agronomic performance, biochemical responses (accumulation of osmoprotectants, carotenoid stability), and mineral status, particularly the accumulation of manganese (Mn^{2+}).

Our objective is to determine how the nitrogen source modulates water stress tolerance and to identify reliable phenotypic markers for breeding programmes: (i) carotenoid preservation as an indicator of photosynthetic protection, and (ii) specific Mn^{2+} accumulation in stems as a marker of ionic adaptation.

MATERIALS AND METHODS

***Rhizobium Strain*:** This experiment investigates the effect of water deficit on the multiplication of *Rhizobium* strain B95. The primary objective is to determine the strain's tolerance to water stress levels that will later be applied to inoculated plants. *Rhizobium* strain B95 was obtained from the National Center for Scientific and Technical Research (CNRST). Due to the lack of prior data on its drought response, the growth of this bacterium was evaluated by measuring the optical density at 600 nm after three days of incubation with mechanical agitation at 120 rpm. Bacterial proliferation was monitored in 200 mL flasks containing 50 mL of autoclaved liquid YEM medium (Vincent, 1970), previously autoclaved at 120°C for 20 minutes. Different levels of water stress treatments were created by varying the concentrations of PEG 6000 added (Table 1).

Table 1. Concentration of PEG 6000 (in % w/v) to create in vitro the equivalent of the stresses studied in relation to field capacity.

Field capacity	Concentration of PEG 6000 (%/V)
1.0	0,9 %
0.9	1,5 %
0.8	2,1 %
0.7	3,0 %
0.6	4,7 %
0.5	9,0 %

***Plant matériel*:** The choice of the two varieties was based on the results of previous experiments, which demonstrated that chickpea varieties supplied with nitrate as a nitrogen source can tolerate severe drought stress at 50% of field capacity. Two varieties with opposite behaviour (Rizki, less sensitive, and Bouchra, more sensitive) were cultivated in symbiotic association with *Rhizobium* strain B95 under different irrigation regimes (100%, 90%, 80%, 70%, 60% and 50% of field capacity).

***Experimental protocol*:** The experiment was conducted in a plastic greenhouse, under semi-controlled conditions, at the Faculty of Science and Technology in Mohammedia. The plants are grown in pots filled with inert sand (1.2 kg per pot). Before sowing, the pots containing sand inoculated with *Rhizobium* strain B95 are watered daily for one week with a nutrient solution (Bargaz et al., 2011; Krouma, 2023), containing 2mM of urea as the nitrogen source. The volume of irrigation provided depends on the intensity of each stress studied (100%, 90%, 80%, 70%, 60% and 50% of field capacity). This treatment was considered necessary to ensure that water stress could act, on the one hand, on the

proliferation of rhizobial bacteria in the soil before plant establishment, and on the other hand, on the processes of association between the macro and micro symbionts, from germination onwards. For each variety, seeds of the same size, after disinfection and inoculation, were sown at a rate of 4 per pot. Upon emergence, a single seedling per pot was maintained in culture until the end of the experiment. For each treatment, the plants were irrigated with a defined volume of nutrient solution to establish the drought stress. The duration of the experiment was 49 days.

Analytical methods

***Agronomic and physiological parameters*:** The morphological parameters evaluated were: length and number of secondary roots, stem height, leaf area, number of leaves, dry mass of each organ, and water content. Measurements were taken at harvest time, using standard methods in plant physiology.

***Biochemical parameters*:**

***Determination of chlorophyll pigments*:** Pigment extraction was performed using a modified version of (Arnon, 1949). The chlorophyll a, chlorophyll b, and carotenoid content (mg. g^{-1}) was calculated using the (Lichtenthaler & Wellburn, 1983)

***Proline determination*:** The method used for proline determination was that of (Monneveux & Nemmar, 1986). The optical density (OD) was measured using a spectrophotometer at 528 nm. The values obtained were converted into proline content according to the calibration curve equation and expressed in mg g^{-1}.

***Solubles sugars determination*:** For the measurement of soluble sugars, we opted for the method described by (DuBois et al., 1956). Optical density is measured at a wavelength of 485 nm. The values obtained are converted into soluble sugar content according to the calibration curve equation and expressed in mg g^{-1}.

Mineral analysis

***Nitrogen determination*:** The total nitrogen content was determined using the Kjeldahl method. The results are expressed as % of nitrogen.

***Mineral determination*:** The mineral elements (P, K^+, Ca^{2+}, Mg^{2+}, Na^+, Fe^{2+}, Zn^{2+} , and Mn^{2+}) from each organ were extracted by acid digestion using the modified HNO_3/H_2O_2 method (Pequerul et al., 1993). The extracts obtained after mineralisation were diluted and then quantified by Agilent 7800 ICP-MS optical emission spectrometry (Technologies International, Tokyo, Japan).

***Statistical analysis*:** Statistical analyses of the different variables were performed using analysis of variance (ANOVA). Comparisons of means were performed using Tukey's test. All statistical analyses were performed using IBM SPSS Statistics Software for windows version 23.0 (IBM Corporation, Armonk, NY, USA). Results from the ANOVA and tukey post hoc test were considered statistically significant at ($p < 0.05$). Graphical analyses were performed using GraphPad Prism software version 9.5.0 (730) for windows (Graphpad Software, San Diego, California USA).

RESULTS

***Effect of drought stress on the growth of Rhizobium strain B95*:** The growth of *Rhizobium* strain B95 was estimated by optical density after 72 hours of culture in (YEM) medium under different intensities of drought stress. The results presented in Table 2 show that, within the range of applied stress levels, water deficit had no apparent effect on the growth of *Rhizobium* strain B95.

Table 2. Variation in optical density (OD) per mL of rhizobial strain B95 as a function of different concentrations of PEG 6000 (in % w/v), simulating in vitro the water stress levels corresponding to the field capacity treatments applied in vivo. Each value represents the mean of three replicates.

Field capacity	O.D 600 nm
Control = without PEG	1,095
100 % ≡ (PEG 6000 = 0,9 %)	1,079
90 % ≡ (PEG 6000 = 1,5 %)	1,075
80 % ≡ (PEG 6000 = 2,1 %)	0,958
70 % ≡ (PEG 6000 = 3,0 %)	0,953
60 % ≡ (PEG 6000 = 4,7 %)	0,910
50 % ≡ (PEG 6000 = 9,0 %)	0,850

Effect of drought stress on agronomic and physiological parameters

***Plant status*:** After 49 days of treatment, plants irrigated with a solution containing mineral nitrogen showed no symptoms of toxicity. However, apart from slight stunting, there was no difference between water-stressed and control plants.

In the presence of *Rhizobium* strain B95, plants from both symbiotic associations exhibited normal size and maintained green leaves when irrigated at 100% field capacity. Increasing water stress led to progressive stunting. Under the most severe stress conditions (60% and 50% FC), the older leaves showed discoloration and turned yellowish.

The number and size of nodules decreased progressively with increasing drought stress, reaching a marked reduction at 70% FC. In the latter case, most of the nodules appeared whitish in colour (Figure 1), suggesting an inhibition of leghemoglobin synthesis.

Meanwhile, the most severe stresses, 60% and 50% of FC, completely suppressed nodulation in both varieties (Figure 2). Therefore, the yellowing of older leaves observed in Fix plants can be attributed to the absence of nodules, which are responsible for reducing atmospheric nitrogen into $NH4^+$. Considered separately, the two partners in the symbiosis exhibit markedly different degrees of tolerance: the host plant is sensitive to water stress, with tolerance limits barely

exceeding 70% of field capacity, whereas the *Rhizobium* strain B95 is highly tolerant, as its multiplication is only slightly affected even under the most severe stress (50% of field capacity).

Figure 1. Whitish colouring of nodules formed under drought stress of 70% of field capacity in both varieties.

Figure 2. Suppression of nodules in both chickpea varieties at 60% and 50% of field capacity.

***Leaves and stems dry matter*:** The results presented in Figure 1 clearly show that, in the absence of drought stress, the dry masses of leaves and stems were highest across both treatments (Nit and Fix). Differences between varieties and nitrogen sources were not very pronounced. However, in both chickpea varieties studied (Rizki and Bouchra), leaves and stems dry matter decreased progressively with increasing severity of water deficit applied. Nevertheless, variations in stress responses were recorded depending on the organ, variety, and nitrogen source.

Regardless of the nitrogen source, reductions in the dry mass of aerial organs were proportional to the intensity of the water stress applied, with a more pronounced effect on leaves than stems, particularly in the Bouchra. Leaf sensitivity is probably due to a strategy implemented by the plant to maintain adequate root growth for good water uptake.

In the presence of mineral nitrogen, Rizki appeared more tolerant to drought stress than Bouchra, as it maintained higher dry mass for its aerial organs even under severe water stress. For exemple, at 50% of field capacity, the dry masses of Rizki leaves and stems were **6.25%** and **37.5%** greater than those of Bouchra, respectively, indicating its better adaptation to the most severe water deficit.

However, Bouchra's sensitivity was attributed to a significant decrease in the dry mass of its aerial organs (leaves: -39.22% and stems: -47.37%) as soon as stress equivalent to 80% of field capacity is applied.

In symbiotic association, the comparison between the two varieties will be limited to stresses ranging from 90% to 70% of FC. Since under more severe stress (60% and 50% of FC), neither variety formed nodules. In both cases, Plant biomass was derived exclusively from the seed nitrogen and the urea supplied to promote the establishment of symbiosis. With the B95 strain, Rizki maintained growth by producing a significant dry mass, even under moderately severe water stress (70% of field capacity). In contrast, Bouchra recorded significant reductions (-21%) in above-ground biomass, even under the most moderate stress levels (90% and 80% of field capacity).

Based on our findings, it is clear that the symbiotic association between Rizki and Rhizobium strain B95 alleviated the effects of water deficit observed in the other association (Bouchra * Rhizobium strain B95). In this regard, Rizki's tolerance in symbiotic association is likely due, on the one hand, to the maintenance of nitrogenase activity, the enzyme responsible for reducing atmospheric nitrogen, ensuring internal nitrogen availability, and, on the other hand, to better specificity with its rhizobial partner. Therefore, the identification of a drought stress-tolerant symbiosis may play a key role in improving the sustainability of legume production systems in arid and semi-arid areas.

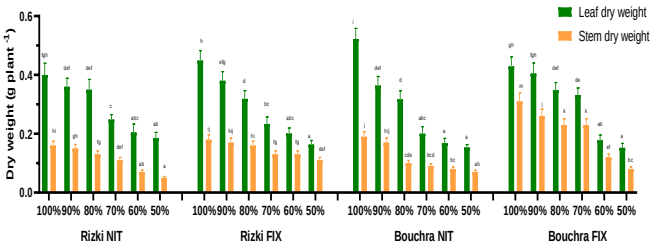

Figure 3. Effect of water stress on leaves and stems dry weight in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Number of leaves and leaf area*:**The results revealed a clear difference in the behaviour of the two varieties depending on the type of treatment applied. With mineral nitrogen, the most severe water stress (50% FC) reduced the average leaf area and number of leaves by **59.45%** and **13.25%** respectively, in Rizki plants. Under the same conditions, the reductions in

average leaf area and number of leaves in Bouchra were **86.25%** and **13.58%** respectively (Fig. 4).

In symbiotic association with *Rhizobium* strain B95, water stress reduced both parameters in both varieties. In this regard, the reductions in average leaf area and number of leaves were more pronounced in Bouchra, with **- 49.59%** and **-13.93%** respectively (Fig. 4).

Regardless of the nitrogen source, it is clear that drought stress affects total leaf area more than the number of leaves produced, indicating a more pronounced effect on extension than on meristematic activity.

Therefore, this significant restriction of photosynthetic area is a strategy adopted by stressed plants, regardless of the nitrogen source, to reduce their potential evapotranspiration area.

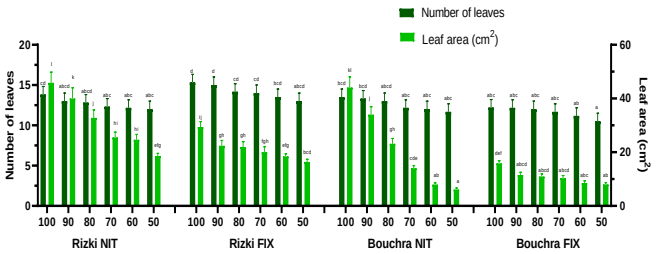

Figure 4. Effect of water stress on the number of leaves and leaf area (cm²) in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Stem height*:** Under full irrigation (100% of field capacity), the varietal effect had no impact on stem height regardless of the nitrogen source. However, under water stress, stem height decreased proportionally to stress severity in both varieties, with a more pronounced effect in Bouchra (Fig. 5).

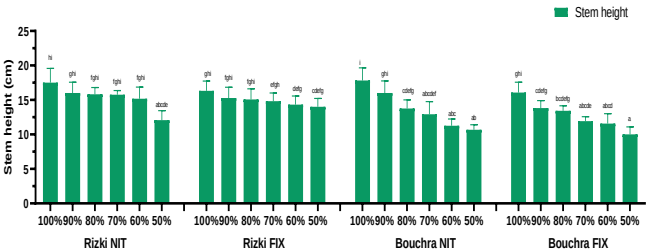

Figure 5. Effect of water stress on stem height (cm) in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

This is because, in Nit plants subjected to stress at 50% of field capacity, stem height decreased by 30.95% and 40.19% in Rizki and Bouchra, respectively. Similarly, in Fix plants, the decreased recorded under 70% field capacity stress were -9.18% and -25.91% in Rizki and Bouchra, respectively. This result clearly shows that water stress reduced the rate of multiplication of apical meristematic cells in the stem under both nitrogen sources, with a stronger inhibition in Bouchra than in Rizki.

***Root system development: length, secondary root formation, and dry biomass*:** Compared to the controls, the root system length of Rizki decreased proportionally to the intensity of water stress applied. Reductions reached -70.44% (50% FC) and -32.72% (70% FC), in Nit and Fix plants Respectively. For secondary roots, except of the 90% field capacity, their number decreased according to the intensity of water stress regardless of the nitrogen source, with reductions of -28.92% (50% FC) and -4.01% (70% FC) in Nit and Fix plants, respectively. However, under 90% field capacity, the number of secondary roots increased by +11.66 % and +20.57 % in Nit and Fix plants, respectively. In general, these two parameters are more affected in Nit than in Fix plants.

Regardless of nitrogen source and variety, root dry weight decreased with the severity of the stress applied. In Nit plants grown under 50% of FC, the reduction in root dry matter production is -70.10% and -79.28% in Rizki and Bouchra, respectively. At 70% FC in plants, similar variations were observed as in Nit plants, with a pronounced effect on the symbiotic association between Bouchra and *Rhizobium* strain B95.

In Bouchra, variations in these three parameters followed patterns comparable to those of the other variety under drought stress, regardless of nitrogen source. However, its root system remained less developed than Rizki across all the treatments.

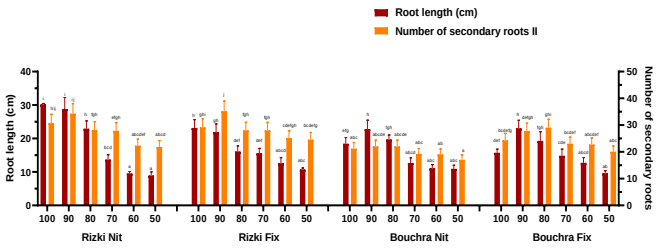

Figure 6. Effect of water stress on root length and number of secondary roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Based on these findings, it is clear that symbiosis reduced the effect of stress on root establishment and development,

thereby improving water supply and promoting nodulation by increasing the number of infection sites for the *Rhizobium* strain. These adaptive mechanisms were more pronounced in Rizki Fix plants.

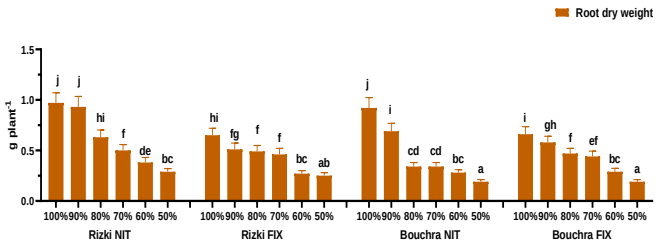

Figure 7. Effect of water stress on root dry weight of two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ

Water Content: In the control condition (100% of field capacity), leaves and stems' water content in Nit plants of both varieties, Rizki and Bouchra, was similar, with better hydration of leaves (6 ml/g dry weight) than stems (5 ml/g dry weight). As drought stress intensity increased, water content progressively declined in both organs. However, in Rizki, this reduction was relatively less pronounced up to 70% FC. But it became more pronounced under severe water regimes (60% and 50% FC). For example, at 50% field capacity, water content decreased by 34.33% and 24.95% in the leaves and stems, respectively.

For Bouchra, drought stress caused a rapid and linear decrease in water content from the lowest stress intensity (90%). At 50% of field capacity, this reduction was more pronounced in the leaves and stems, with a decrease of -50% and -60% compared to the control.

In symbiotic association and under full irrigation, the water content of leaves and stems of both varieties, Rizki and Bouchra, was similar, with better hydration of the leaves (5 ml/g dw) compared to the stems (4 ml/g dw). Overall, the aerial organs of Fix plants were less hydrated (-16.66%) than those of Nit plants.

Under drought stress, variations in water content of the aerial organs in Fix plants, especially leaves, were not very noticeable but remained similar to those of Nit plants up to 70% of field capacity. Therefore, it seems that symbiosis partially mitigates the effect of stress, especially in photosynthetic organs.

Comparing the responses of the two varieties, it is clear that Rizki showed a better water supply to its aerial organs, even under severe drought stress (50% FC). This resilience could be due to a good regulation of water homeostasis, in particular through effective control of transpiration or adequate root uptake.

In contrast, Bouchra showed high sensitivity to drought stress, with a rapid decline in water content even under the lowest levels (90% FC). These findings suggest that the mechanisms implemented by this variety are less effective in maintaining good water absorption or that its root tissues are highly sensitive to water deficit.

In symbiotic association, the effect of drought stress was attenuated in both varieties compared with Nit plants. However, this protective effect was more pronounced in Rizki than in Bouchra. Rizki's moderate tolerance may be attributed to the effectiveness and efficiency of its symbiotic association with its rhizobial partner, allowing for better allocation of energy resources and better osmotic regulation. On the other hand, Bouchra's sensitivity could be linked to its low symbiotic efficiency or to the high sensitivity of its nodules to water stress.

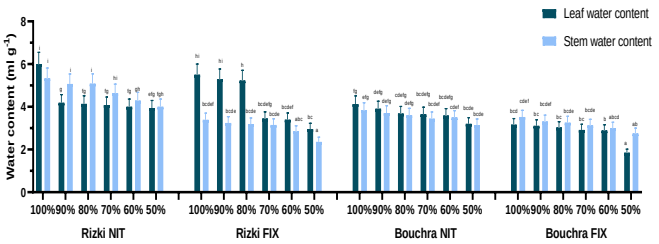

Figure 8. Effect of water stress on the water content of leaves and stems in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Parameters related to nodulation*:** Under control conditions, both chickpea varieties (Rizki and Bouchra), inoculated with *Rhizobium* strain B95 developed functional nodules on their root systems, indicating the establishment of effective symbiosis; however, the number of nodules formed was slightly higher in Rizki. This slight superiority in terms of nodulation observed in Rizki may be due to a better genetic compatibility with the *Rhizobium* strain B95 used or to the secretion of a large amount of flavonoids that promote nodulation induction (Zahran, 1999).

Figure 7 also shows that water stress had an inhibitory effect on the nodulation process, reducing the number of nodules formed as soon as the lowest stress level is applied (90% of field capacity), indicating the sensitivity of the nodulation process to reduced water availability. At 70% of field capacity, the number of nodules reduced by 50% and 65% respectively in Rizki and Bouchra compared to the controls. However, under severe stress conditions (60% and 50% of field capacity), the roots of both varieties did not form any nodules (Figure 2). This inhibition of nodulation shows that

symbiotic nitrogen nutrition is considerably sensitive to drought stress than mineral nutrition.

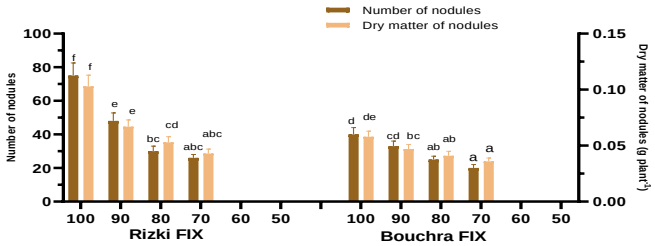

Figure 9. Effect of water stress on the nodule number and nodule dry matter in two varieties, Rizki and Bouchra, under symbiotic nutrition conditions (FIX).

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Effect of drought stress on biochemical parameters

***Chlorophyll pigments*:** In the absence of drought stress, the chlorophyll *a* content of Nit plants of Rizki recorded high values (+34.09%) compared to those of Bouchra. However, the content gradually decreased, increasing stress in both varieties. In contrast, the decline was evident in Bouchra as soon as the lowest stress level (90% FC) was applied. At 50% field capacity, the values measured were 0.19 and 0.13 mg/g DM for Rizki and Bouchra, respectively (Fig. 10).

Under full irrigation, chlorophyll *b* levels were lower than chlorophyll *a* in both varieties. Under drought stress, the decreases were similar to those of chlorophyll a. At 50% of field capacity, the reductions were -60% and -75% in Rizki and Bouchra, respectively (Fig. 10). For carotenoids, levels only decreased about -60% in Nit Rizki plants, irrigated at 80% of field capacity. After that, the values remained relatively stable even as the intensity of water stress increased. In contrast, carotenoid content in Bouchra declined proportionally to stress intensity (Fig. 10).

Our results clearly showed that Rizki maintained its chlorophyll a and b concentrations even under severe water stress (50% FC). This performance could be attributed to better regulation of pigment biosynthesis or less enzymatic degradation under stress. In contrast, a decrease in chlorophyll pigment content was observed in Bouchra even at the lowest stress intensity (90%). Its high sensitivity can be explained by the inefficiency of the mechanisms put in place to protect and maintain the synthesis of these pigments or from the alteration of biosynthetic processes initiated by the slightest decrease in water supply.

In symbiotic association, chlorophyll *a* levels were lower than those observed in Nit plants, with a superiority of approximately +50% in Rizki Fix plants. In response to drought stress, decreases in content were proportional to stress severity, with a more pronounced effect at 70% of field capacity, especially in Bouchra Fix plants (Fig. 10). For chlorophyll *b*, levels in Fix plants of both varieties under full irrigation were similar to those of Nit plants. Although the contents were higher in Rizki Fix plants, Under drought stress, contents in plants of both symbiotic associations followed the same variations as those of chlorophyll *a* (Fig. 10).

For carotenoids, the contents recorded a reduction of approximately -50% only in Fix plants of Bouchra subjected to the lowest water stress (90% FC). At 70% of field capacity, the decreases were very significant in Bouchra (-78.46%). In Rizki, however, the levels remained relatively stable, with no apparent effect (Fig. 10).

In conclusion, symbiotic association partially reduced the effect of drought stress in both varieties. However, this protective effect was more pronounced in Rizki. This can be explained by the effectiveness and efficiency of its symbiotic association in ensuring better allocation of energy, nitrogen, and magnesium resources for chlorophyll pigment biosynthesis. As well as to the stability of its carotenoid content, which plays a fundamental role in protecting chlorophyll from various environmental stresses.

Therefore, carotenoid content can be used as a biochemical parameter for predicting the tolerance of a symbiotic association in legumes.

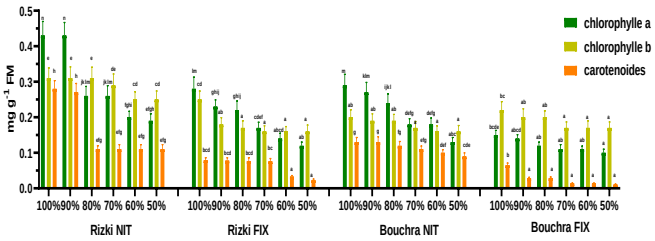

Figure 10. Effect of water stress on chlorophyll pigments (a, b, carotenoids) in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Proline content: Regardless of the treatment, proline levels increased with the intensity of drought stress applied, in both leaves and roots, with the highest values recorded in plants irrigated at 50% of field capacity (Fig. 11). In general, the levels of this amino acid in leaves and roots were higher in Rizki than in Bouchra, across all treatments

This superiority of Rizki may be attributed to its ability to synthesise proline under water deficit, particularly in the leaves, ensuring better allocation of carbohydrates and symbiotically fixed nitrogen to the biosynthesis processes of this amino acid. On the other hand, Bouchra's sensitivity to water stress can be explained either by the inefficiency of its

osmotic regulation mechanisms or by the low availability of photosynthates and reduced nitrogen from nodules, which are necessary for proline biosynthesis.

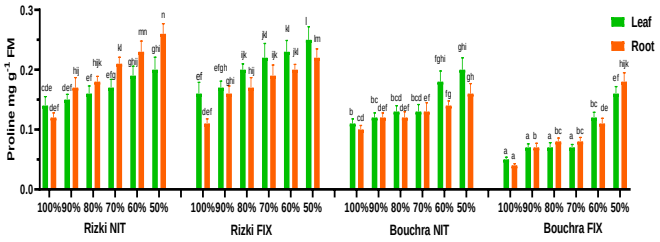

Figure 11. Effect of water stress on proline content in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Solubles sugars*:** Figure 12 shows that soluble sugar content in leaves and stems increased with intensifying water stress, reaching the highest values at 50% of field capacity in the various treatments tested. Under full irrigation conditions (100% FC), the highest content was observed in Fix plants of both varieties. However, under drought stress, the levels were slightly higher in the Nit plants of both varieties.

Comparing the two organs, it is clear that soluble sugar contents were generally higher in leaves than in roots, regardless of treatment or variety.

This result confirms the direct involvement of leaves, exposed to water deficit, in the accumulation of carbohydrates as protective osmoregulators to preserve all the vital functions of photosynthetic plant cells.

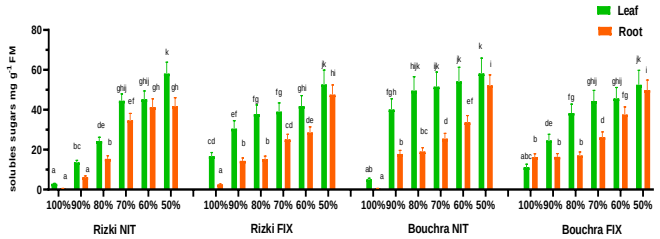

Figure 12. Effect of water stress on soluble sugar content in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Effect of drought stress on mineral content

Leaves, stems and roots

Nitrogen (N): Figure 13 shows that leaves are the organs richest in nitrogen, followed by stems and roots, regardless of the type of treatment and variety studied; however, values were significantly higher in the presence of mineral nitrogen. between varieties, Rizki organs consistently contained more mineral nitrogen than those of Bouchra, under both mineral nutrition and symbiotic association conditions. At 100% FC, the decrease in nitrogen content in Fix compared to Nit plants was similar in the leaves and roots of both varieties.

However, under drought stress with mineral nitrogen, the total nitrogen content of different organs decreased as the intensity of stress increased, with a more pronounced effect on the stems. For example, at 50% of field capacity, the reduction in nitrogen content in these organs was -57.86% and -76.62% in Rizki and Bouchra, respectively.

Similarly, in symbiotic association, the highest reductions, varying between -80% and -90% depending on the organ of each variety, were recorded under 60% and 50% of field capacity. In this regard, it should be noted that the roots of plants subjected to these two treatments did not form nodules. As a result, the measured contents essentially correspond to the urea applied during the first two weeks of culture to promote nodulation and that of the seeds. For moderately severe stress, the decreases were proportional to their severity in both types of symbiotic associations, with a slightly marked effect on the organs of Bouchra. At 70% FC, the decrease in atmospheric nitrogen reduction in the nodules is -26.37% and -48.57% in Rizki and Bouchra, respectively. Therefore, the symbiotic association between Bouchra and *Rhizobium* appeared to be very sensitive to moderate water stress.

Under drought stress, leaves always retain the highest nitrogen content, which could be linked to a survival strategy to maintain the photosynthetic activity necessary for biomass synthesis and plant growth. In contrast, roots showed a significant decrease, favouring redistribution to the aerial parts. The variations observed between Rizki and Bouchra can be attributed to genetic differences in their ability to tolerate drought stress and interact with their rhizobial partner.

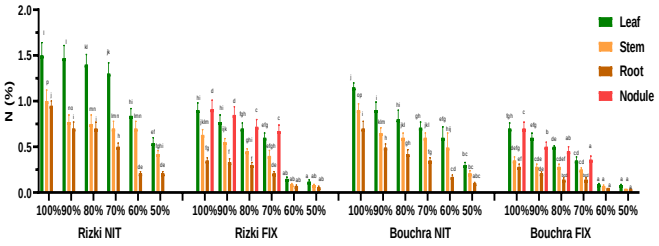

Figure 13. Effect of water stress on total nitrogen content in four organs: leaves, stems, roots, and nodules in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

It appears that the relative resistance of the symbiotic association with Rizki is mainly due to its ability to reduce atmospheric nitrogen more efficiently, rationalise its distribution between its organs and optimise its use under water deficit. These findings highlight the importance of selecting varieties or symbiotic associations with mechanisms adapted to nitrogen supply under unfavourable conditions to improve crop productivity.

***Phosphorus (P)*:** Water stress, caused by a reduction in field capacity (from 100% to 50%), significantly affected phosphate absorption and distribution in the plant's various organs. In general, aerial organs were less affected than roots. This is because reductions in Nit plant leaf content were only noticeable in Rizki and Bouchra when the most severe drought stress was present. Similarly, for Fix plants, the fluctuations were similar to those of Nit plants (Fig.14).

Therefore, Rizki's relative tolerance, regardless of the nitrogen source, is attributed to its ability to maintain adequate phosphate absorption for its foliar metabolism under a water regime of 60% FC. In this case, Rizki's performance, in symbiotic association with its rhizobium, can be attributed to good root absorption, better resource distribution, and potential nitrogen-phosphorus synergy.

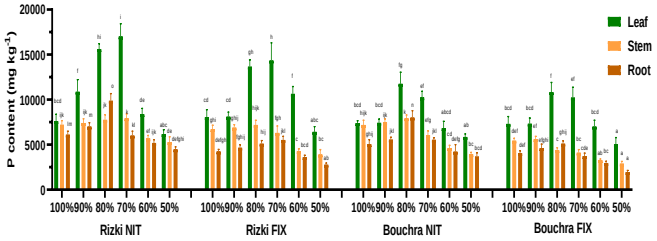

Figure 14. Effect of water stress on phosphorus content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX .

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Potassium (K^+)*:** The results show that drought stress significantly affected potassium levels in the leaves, stems, and roots of both chickpea varieties, with differences related to variety and nitrogen source. In Rizki, the levels of P in all organs enhanced with increased stress, with the highest values recorded under the most severe water regime (Fig. 15). Similarly, for Bouchra, the variations in the aerial organs were similar to those of Rizki. However, in the roots, the levels increased slightly to 80%, then decreased to 60% and 50% of FC.

According to our findings, the moderate tolerance of Rizki to water deficit, regardless of the nitrogen source, is related to its ability to maintain good potassium absorption and accumulation in its root system.

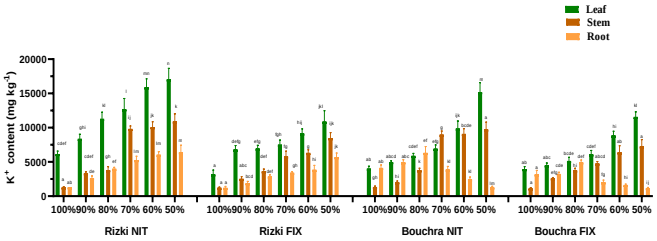

Figure 15. Effect of water stress on Potassium content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Sodium (Na^+): In Rizki, Na^+ levels increased gradually with the intensification of water stress in all organs, regardless of the nitrogen source. However, significantly higher values were observed in the stems and roots, especially in Fix plants. For Bouchra, the levels in the stems and roots of Nit and Fix plants followed the same trends with stress severity as those observed in the other variety (Fig. 14). On the other hand, in Nit plants of this variety, leaf levels showed a slight increase up to 80% FC, then gradually decreased with the severity of stress, recording a value similar to that of the control at 50% FC. For Fix plants of Bouchra, the decreases in levels were very high as stress increases.

Based on these results, Rizki's tolerance may be linked to the effectiveness of its physiological mechanisms involved in the absorption, xylem transport, and Na^+ accumulation in its photosynthetic organs. This is because osmotic adjustment with sodium is vital under drought stress.

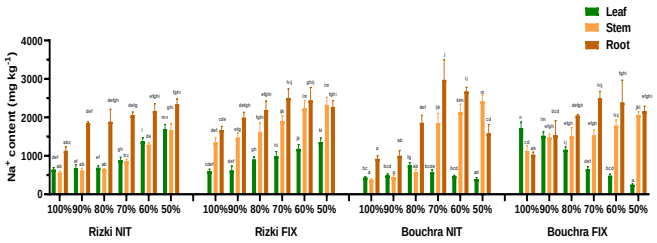

Figure 16. Effect of water stress on Sodium content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

***Calcium (Ca^{2+})*:** Calcium (Ca^{2+}) levels in aerial organs increased in line with stress intensity in Nit plants up to 80% of field capacity, then gradually decreased. At 50% FC, leaf

content is reduced by 55% and 64% in Rizki and Bouchra, respectively. In contrast, in Fix plants, the reductions were proportional to the intensification of stress. Regardless of Nitrogen source, root content increased with stress severity, recording the highest values under a water regime of 70% FC. However, in these underground organs, reductions were only observable in plants subjected to the most severe stress (Fig. 18).

This result clearly showed the role of Ca^{2+} in maintaining membrane integrity and, in particular, in the functioning of absorption systems.

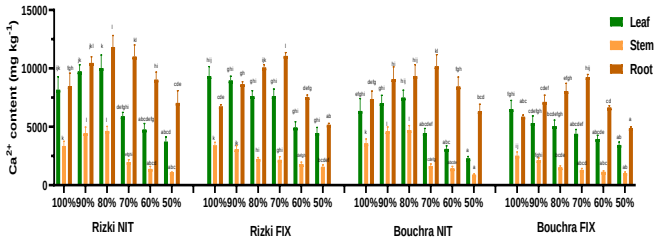

Figure 18. Effect of water stress on Calcium content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Magnesium (Mg^{2+}): Regardless of nitrogen source, magnesium (Mg^{2+}) levels in stems and roots increased with stress intensity, except for the 50% FC water regime. In photosynthetic organs, levels tended to decrease under symbiotic atmospheric nitrogen conditions and increased in moderately stressed nitrate environments. In the latter case, the highest levels were obtained under irrigation regimes of 80% and 70% of field capacity in Bouchra and Rizki, respectively (Fig. 19).

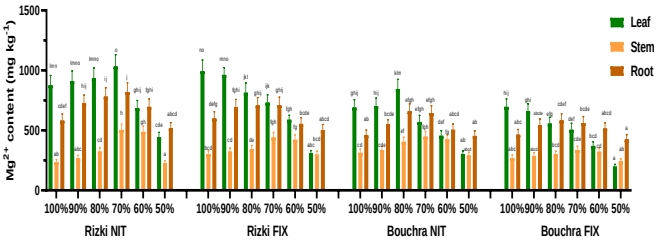

Figure 19. Effect of water stress on Magnesium content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Iron (Fe^{2+}): In Rizki Nit plants, iron levels increased with intensifying water stress in the stems and roots. However, in the leaves, the contents only decreased when stress reached 60% FC. In Fix plants of the same variety, root levels followed similar variations to those of Nit plants; leaf and stem contents decreased by -36.28% and -6.25% respectively, under 70% and 60% FC (Fig. 20). In Bouchra, stem levels tended to increase with stress intensity in Nit plants, but remained almost stable in Fix plants. Regardless of the nitrogen source, variations in leaf and root levels as a function of drought stress intensity were similar in this variety (Fig. 20).

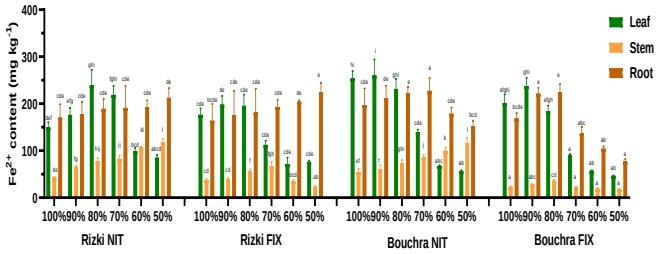

Figure 19. Effect of water stress on iron content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Zinc (Zn^{2+}): In general, the variations in Zn^{2+} in all organs were similar to those in Mg^{2+} observed in Nit and Fix plants of both varieties. However, it appears that the absorption and xylem transport of this bivalent cation to photosynthetic organs were inhibited as soon as the lowest stress was applied in Fix plants of both varieties. In contrast, in Nit plants, this reduction was only detectable for stresses greater than or equal to 70% of field capacity (Fig. 20).

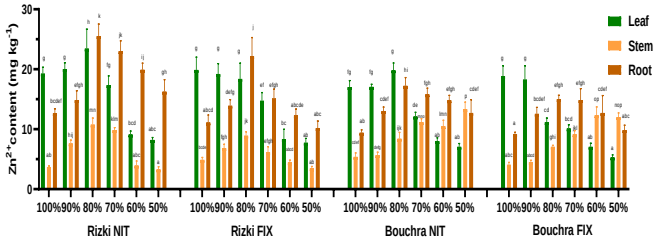

Figure 20. Effect of water stress on Zinc content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Manganese (Mn^{2+}): Regardless of variety, the results showed that the accumulation levels of this bivalent cation in Nit plants increased with the intensification of moderate stress; then decreased to 70% and 60% of field capacity in Bouchra and Rizki, respectively. However, in Fix plants, decreases were noted in the leaves even at the lowest stress levels. As for the roots, the reducing effect was only observable in the presence of the most severe stress (60% and 50% of FC). At the stem level, increasing content as stress intensified was recorded only in Rizki plants, with a slight superiority of Nit plants. In contrast, in the other variety, the content showed no variation either with the nitrogen source or with the severity of drought stress (Fig. 21).

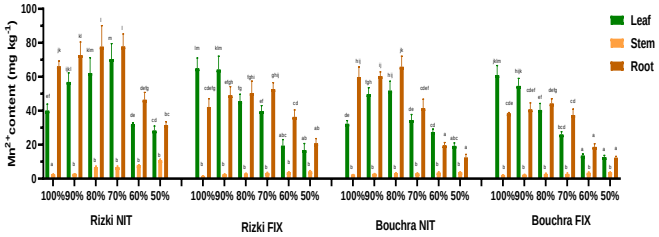

Figure 21. Effect of water stress on Manganese content in three organs: leaves, stems, and roots in two varieties, Rizki and Bouchra, under two types of nitrogen nutrition: NIT and FIX.

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

Ionic status of nodules

Nitrogen: Table 1 shows that Rizki nodules were generally richer in nitrogen under all levels of water stress. This difference was more pronounced at 100% and 90% field capacity, where Rizki nodule contents were higher than those of Bouchra. As drought stress severity increased, nitrogen concentrations decreased in both symbiotic associations. At 70% field capacity, the reduction is -26.37% and -48.57% for Rizki and Bouchra, respectively.

Therefore, the symbiotic association between Rizki and the *Rhizobium* strain B95 seems to be better adapted to water deficit conditions, which could be related to its ability to maintain effective symbiotic activity even under unfavourable conditions.

Therefore, the nitrogen concentration in nodules represents an important indicator of the maintenance of nitrogenase reductase activity under water stress. This parameter can be used in the selection of *Rhizobium* strains to improve the sustainability and productivity of chickpea crops in semi-arid environments or drought-prone areas.

***Phosphorus*:** Table 1 shows that Rizki nodules were generally richer in phosphate at all levels of water stress. However, the difference between the two symbiotic associations was less marked than that observed for nitrogen. At 70% FC, the reductions observed were very similar, ranging from -24 to -25%, indicating a similarity in their response to stress.

The gradual decrease in phosphorus content with increasing drought stress highlights the vital role of water in the

Table 3. Effect of water stress on mineral content in nodules in two varieties, Rizki and Bouchra.

Minerals	Treatment/ Variety	100%	90%	80%	70%	60%	50%
N content (%)	Rizki	0.91 ± 0.095^{d}	0.85 ± 0.089^{d}	0.72 ± 0.075^{c}	0.67 ± 0.069^{c}	-	-
	Bouchra	0.7 ± 0.072^{c}	0.5 ± 0.052^{b}	0.45 ± 0.049^{ab}	0.36 ± 0.037^{a}	-	-
P content (mg/kg)	Rizki	6300 ± 690^{c}	5820 ± 640^{bc}	5500 ± 600^{abc}	4770 ± 520^{ab}	-	-
	Bouchra	6000 ± 660^{c}	5750 ± 630^{bc}	5200 ± 570^{abc}	4500 ± 490^{a}	-	-
K content (mg/kg)	Rizki	34000 ± 4100^{d}	32700 ± 3940^{cd}	$29500\pm3550^{a-d}$	25600 ± 3080^{ab}	-	-
	Bouchra	32000 ± 3860^{bcd}	$30200\pm3640^{a-d}$	27300 ± 3290^{abc}	23800 ± 2870^{a}	-	-
Ca content (mg/kg)	Rizki	7900 ± 870^{a}	8300 ± 910^{a}	8600 ± 950^{a}	8900 ± 980^{a}	-	-
	Bouchra	7600 ± 840^{a}	7900 ± 870^{a}	8200 ± 900^{a}	8500 ± 930^{a}	-	-
Na content (mg/kg)	Rizki	1300 ± 143^{a}	1800 ± 198^{b}	2700 ± 297^{c}	3800 ± 418^{d}	-	-
	Bouchra	1000 ± 110^{a}	1300 ± 143^{a}	1900 ± 209^{b}	2800 ± 308^{c}	-	-
Mg content (mg/kg)	Rizki	470 ± 57^{b}	430 ± 52^{ab}	400 ± 48^{ab}	380 ± 46^{ab}	-	-
	Bouchra	450 ± 54^{ab}	400 ± 48^{ab}	380 ± 46^{ab}	360 ± 43^{a}	-	-
Fe content (mg/kg)	Rizki	$119\pm14,34^{c}$	$114\pm13,74^{c}$	$106\pm12,77^{abc}$	$89\pm10,72^{ab}$	-	-
	Bouchra	$117\pm14,1^{c}$	$112\pm13,5^{bc}$	$102\pm12,29^{abc}$	$85\pm10,24^{a}$	-	-
Zn content (mg/kg)	Rizki	$44\pm5,3^{d}$	$41\pm4,94^{cd}$	$37\pm4,46^{abcd}$	$31\pm3,74^{ab}$	-	-
	Bouchra	$42\pm5,06^{cd}$	$39\pm4,7^{bcd}$	$35\pm4,22^{abc}$	$29\pm3,49^{a}$	-	-
Mn content (mg/kg)	Rizki	$39\pm4,7^{d}$	$36\pm4,34^{bcd}$	$33\pm3,98^{abcd}$	$29\pm3,5^{ab}$	-	-
	Bouchra	$37\pm4,46^{cd}$	$34\pm4,1^{abcd}$	$31\pm3,74^{abc}$	$27\pm3,25^{a}$	-	-

The bars represent the mean of six replicates ± standard deviation. Different letters indicate a significant difference ($p<0.05$) according to Tukey's post hoc test. Comparisons are made separately for each organ.

mobilisation and utilisation of essential nutrients in symbiotic nodules.

***Mineral cations*:** In the absence of water stress, the difference between the two symbiotic associations lies in the ionic status of their atmospheric nitrogen-fixing organs: Rizki's nodules were more loaded with K+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Mn^{2+} , and Na^{+} than those of Bouchra. Except for Ca^{2+} and Na^{+}, the levels of other divalent cations tended to decrease with the severity of stress in both associations; however, this decrease is particularly pronounced in Bouchra. On the other hand, Ca^{2+} and Na^{+} concentrations increased with decreasing field capacity, with a higher level of accumulation in Bouchra (Table 3). At 70% of field capacity, Na^{+} levels increased by +192.31% and +180% in Rizki and Bouchra, respectively.

This excessive sodium overload in the nodular tissues of both varieties highlights the effect of drought stress on their ionic status and underlines the need to study the specific mechanisms of stress tolerance in different Rhizobium strains.

DISCUSSION

***Discussion of agronomic and biochemical parameters*:** This study shows that water stress applied from sowing to flowering affected growth, nodulation, and nitrogen fixation parameters. The sensitivity of chickpeas is confirmed, as dry matter production decreased even when the lowest level of stress is applied. The R/PA ratio increased with stress intensity, indicating a more pronounced effect on aerial organs. This result is consistent with those of other studies (Ramamoorthy et al., 2017; Medeiros et al., 2024).

Leaf area varied in line with dry matter weight; however, the effect of water stress was more pronounced on leaf expansion than on the formation of new organs. Our results corroborate those of (Chaves et al., 2003), who showed that plants grown under water stress maintain their active photosynthetic organs by reducing their leaf area to limit water loss. (Seufert et al., 2019) attributed this leaf response in stressed plants to a combined limitation of water and assimilable nitrogen. Similarly, several studies have shown that coordination between nitrogen signalling and water stress responses plays a key role in regulating leaf expansion via hormones such as abscisic acid (ABA) and cytokinins (Peuke & Jeschke, 1993; Gholipoor et al., 2010).

Under water stress, plants activate osmotic mechanisms to maintain intercellular osmotic pressure, favourable for water uptake. In this regard, tolerant plants synthesize more osmoregulators, such as proline and soluble sugars, to compensate for water loss, while sensitive plants are characterised by their limited capacity to produce these protective substances (Krishnamurthy et al., 2010). Tolerant plants also preserve intracellular water by reducing transpiration through stomatal closure (Vadez et al., 2012a; Nour et al., 2024). Similarly, tolerant varieties frequently develop deeper or more branched root systems than sensitive plants, allowing for better water absorption (Kou et al., 2022). The symbiotic association can improve drought tolerance by supplying fixed nitrogen, thereby reducing dependence on mineral nitrogen uptake by the roots. This enables plants to allocate more energy toward water regulation and the synthesis of protective molecules (Aranjuelo et al., 2015). However, this protective effect was suppressed under severe stress, as nodules themselves became more sensitive and/or fail to form.

Inoculation of two chickpea varieties with *Rhizobium* strain B95 showed that they were able to form nodules under moderate water stress, not exceeding 70% of field capacity. These findings align with those reported by (Vadez et al., 2012a; Kunert et al., 2016), indicating that a 20-30% reduction in soil moisture, significantly impairs chickpea nodulation.

The decrease in nodule mass as stress intensified was consistent with previous studies. (Iqbal et al., 2022). This phenomenon likely resulted from inhibited multiplication of either the bacterial or host cells. Water stress may have directly impaired cell division or indirectly limited it by reducing carbohydrate availability.

Under the most severe water stress conditions (60% and 50% of field capacity), nodulation was completely suppressed in both varieties. This result demonstrates the particular sensitivity of the chickpea * *Rhizobium* symbiosis. This inhibition of rhizobial symbiosis was attributed to be due to a reduction in root exudates (Ladrera et al., 2007), alteration in molecular signals between the two partners (Aranjuelo et al., 2015), and a decrease in potential infection sites for rhizobia, due to reduced root elongation and inhibition of root hair curvature (Serraj et al., 1999). Notably, strain B95 maintained its growth under the various water stress conditions studied.

In this regard, our findings contradict those of (Zahran, 1999), who suggested that nodule suppression resulted directly from water stress affecting the viability and activity of rhizobial bacteria in the rhizosphere. However, the observed differences between the two symbiotic associations may have resulted from genetic variations in stress response, particularly regarding oxidative tolerance or osmo-protection regulation (Krishnamurthy et al., 2010).

The reduction in infection appeared to be attributable to insufficient calcium nutrition, as Ca^{2+} supplementation has been shown to improve root growth and promote the emergence of absorbent hairs (Fang et al., 2024; Yeremko et al., 2025). Our results indicate that Ca^{2+} uptake became limiting at 60% FC.

Data on nodule dry weight showed significant decreases in response to water stress in both chickpea varieties studied. The reductions were 37% and 58% in Rizki and Bouchra, respectively. This low inhibition of nodule development observed in Rizki compared to Bouchra may have been due to the specificity between the two symbiotic partners, the

maintenance of adequate nodular metabolic activity, or more effective antioxidant mechanisms protecting nitrogenase from oxygen- induced inhibition effect, during drought stress. (Ladrera et al., 2007; Aranjuelo et al., 2015).

This behaviour, frequently observed in certain chickpea varieties adapted to arid and semi-arid environments, reflects strategies of resource conservation and reduced dependence on symbiosis under water deficit (Krishnamurthy et al., 2010; Vadez et al., 2012a).

The observed tolerance of Rizki nodules may have resulted from a slight reduction in carbohydrate allocation to the nodules, which serve as essential energy substrates for atmospheric nitrogen reduction (Khatun et al., 2021). The symbiotic association with Rizki maintained adequate nodular osmotic potential to support nitrogenase activity and bacteroid viability (Serraj et al., 1999), as well as sustained root growth necessary for the formation and development of new nodules (Vadez et al., 2012a). Collectively, these findings indicate that the symbiotic association with Rizki employed an active and productive strategy particularly well adapted to arid and semi-arid soils.

Water stress disrupted the symbiotic relationship between legumes and nitrogen-fixing bacteria, resulting in a significant reduction in nodular biomass and a decreased capacity for atmospheric nitrogen fixation (Istanbuli et al., 2025). In contrast, specific genotypes such as IG70384 and IG70410 demonstrated resistance to drought stress. These genotypes maintained better nodulation and exhibited increased nodular biomass under limited irrigation or reduced rainfall (Istanbuli et al., 2025).

In general, water deficit typically stimulated the activity of enzymes responsible for chlorophyll pigment degradation. The tolerance observed in certain plants was associated with their capacity to develop protective mechanisms against these enzymes (Krishnamurthy et al., 2010). Additionally, water stress decreased the biosynthesis of photosynthetic pigments by restricting the availability of essential biosynthetic substrates.

Tolerant plants regulated their metabolism to sustain a minimal and essential pigment synthesis, whereas sensitive plants experienced a rapid reduction in biosynthetic activity (Vadez et al., 2012a). In symbiotic associations, chlorophyll pigment biosynthesis was comparatively less affected (Serraj et al., 1999; Hami et al., 2025), due to the availability of symbiotically fixed nitrogen that enabled greater allocation of energy to photosynthetic processes. Nevertheless, under severe stress, the maintenance of chlorophyll biosynthesis was compromised because nitrogen-fixing activity in the nodules was inhibited (Aranjuelo et al., 2015).

The present results aligned with those of (Vadez et al., 2012a), who demonstrated that certain chickpea varieties can maintain better pigment concentrations under water stress, indicating tolerance. Carotenoids play a critical role in protecting thylakoid lipid membranes from oxidative stress caused by water deficit, particularly in chickpeas, which are extensively cultivated in semi-arid regions (Ashraf & Harris, 2013). Carotenoids, as a major antioxidants within the photosystem, dissipate excess energy as heat (non-photochemical quenching) and neutralize reactive oxygen species (ROS) generated during photoinhibition (Demmig & Adams, 2006). Our results confirmed this protective effect of carotenoids against the degradation of chlorophylls *a* and *b* under different levels of drought stress, particularly in plants associated with *Rhizobium* strain 95. Therefore, atmospheric nitrogen fixation supports more sustainable photosynthetic activity by limiting the generation of ROS and preserving the photosynthetic apparatus. This result suggests that rhizobial symbiosis improves not only nitrogen assimilation but also the biosynthesis of photoprotective pigments by providing the precursors (such as pyruvate and glyceraldehyde-3-P) necessary for the pathways of terpenoid metabolism. Consequently, Rizki's tolerance under both nitrogen regimes can be explained by its ability to maintain high carotenoid levels under water stress thereby protecting its chlorophyll pigments (Ashraf & Harris, 2013). On the other hand, the variations in chlorophyll pigment content observed in Bouchra were similar to those cited by (González, EM et al., 2001), who showed that water deficit induces the activation of enzymes involved in the degradation of chlorophyll pigments. These results demonstrate that nitrogen source influenced water stress tolerance both through nutritional effects and by enabling photoprotective defense mechanisms.

The results indicated that under severe water stress, proline biosynthesis pathways were likely inhibited due to energy deficits in ATP and NADPH or limited nitrogen precursors, particularly glutamate. Mineral nitrogen assimilation depended strongly on soil hydration and root conductance. Reduced soil moisture during severe drought restricted nitrogen availability for amino acid synthesis. Symbiotic nitrogen sources supported sustained proline accumulation, likely by providing a stable supply of organic nitrogen even under moderate to severe stress (Serraj & Sinclair, 2002). These findings were consistent with those of (Serraj & Sinclair, 2002), who reported that biological nitrogen fixation enhances legume resilience to water deficit. Additionally, the genotypic variability observed in this study aligned with previous reports (Kaushal et al., 2013; Kaur & Khanna, 2016).

The observed results for soluble sugars were aligned with existing literature. The increase in soluble sugars represents an adaptive response by plants to maintain cellular homeostasis and protect cellular structures from damage caused by water stress (Farooq et al., 2009). These molecules, such as glucose, fructose, and sucrose, act as protective osmolytes, stabilizing proteins and cell membranes while maintaining cell turgor under stress conditions (Serraj & Sinclair, 2002; Chaves et al., 2009; Amoah & Adu-Gyamfi, 2025). However, leaves, as the main photosynthetic organs, accumulated more soluble sugars, probably to maintain their

metabolic activity and cell viability (Chaves et al., 2009). In contrast, the decreases observed in roots, which are directly exposed to water deficit, appeared to be due to their use in signaling and adaptive growth processes (Lastdrager et al., 2014).

Concerning the effect of the nitrogen source on the response to water stress, it should be noted that symbiotic plants recorded soluble sugar contents nearly identical to those of Nit plants, particularly under moderate stress. In this regard, previous studies showed that symbiotic associations developped better stress tolerance through more effective regulation of stress-related genes and the accumulation of osmolytes (Laranjo et al., 2014).

Similarly, other studies showed that bacteria of the genus *Rhizobium* can produce phytohormones and signalling molecules to improve plant responses to water stress (Zahran, 1999; Graham & Vance, 2003). Regardless of the nitrogen source, it appears that the physiological mechanisms involved in osmoregulatory metabolic pathways are more effective in Rizki (Vadez et al., 2007). Therefore, the rhizobian symbiosis of this variety can be exploited in arid and semi-arid areas where mineral nitrogen is limited or expensive (Zahran, 1999). Analysis of our results revealed a reorganization of ionic homeostasis and nitrogen nutrition in two chickpea varieties, Rizki and Bouchra, in response to water stress, influenced by the source of nitrogen: either mineral or rhizobial symbiosis. This reorganisation reflects divergent adaptive strategies, determined by genetic traits, energy constraints, and complex interactions between nutrients.

For nitrogen, the recorded values showed that leaves were the richest organs, regardless of treatment or genotype. This foliar enrichment in nitrogen was attributed to the preservation of photosynthetic tissues, which are the primary sites of protein enzyme synthesis (e.g., RuBisCO) and chloroplast components essential for biomass production (Taiz, L et al., 2018). The superiority of nitrogen content in the organs of plants fertilized with mineral nitrogen (Nit) compared to plants in symbiotic association (Fix) highlighted the immediate effectiveness of exogenous input, particularly under non-limiting conditions (100% field capacity). However, this difference was partially attenuated in Rizki, suggesting greater efficiency of biological fixation in this variety, although it remained lower than the mineral input.

Regardless of the nitrogen source, Rizki demonstrated superior performance across all treatments due to its intrinsic capacity for efficient nitrogen acquisition, redistribution, and utilization. This efficiency resulted from enhanced root absorption of nitrates through transporters such as NRT2.1, increased assimilation associated with elevated nitrate reductase (NR) and glutamine synthetase (GS) activity, and improved phloem-mediated redistribution of nitrogen from senescent tissues to developing organs (Masclaux-Daubresse et al., 2006).

Nitrogen levels declined progressively in all plant organs as water stress intensified. The magnitude of this decline was determined by both the nitrogen source and the varietal effect. In nit-supplied environments, reduced xylem flow may have limited nitrate (NO_3^-) absorption and translocation, while water stress also decreased the activity of key nitrogen assimilation enzymes (NR, GS) (Serraj & Sinclair, 2002). Additionally, significant protein degradation may have occurred, releasing assimilable nitrogen to active tissues (Masclaux-Daubresse et al., 2006).

In symbiotic plants, the nitrogen measured in plants subjected to water stress of 60% and 50% of FC came exclusively from seed reserves and urea applied during the first two weeks of cultivation. This is because no nodules were observed on the roots of plants of either variety grown under these two water regimes. This inhibition confirmed the high sensitivity of the nodulation process to water deficit (Martin et al., 2023). Even under moderate stress (70% FC), atmospheric nitrogen fixation was significantly reduced in Bouchra (- 48.57%) compared to Rizki (-26.37%).

This difference between the two symbiotic associations showed that the association with Rizki was able to maintain metabolic activity in its nodules. It could adjust transpiration through stomatal regulation or maintain carbohydrate allocation to the roots. All this ensured the survival and physiological activity of the symbiotic bacteria (Serraj et al., 1994) . Despite the generalised decline in all organs, the leaves retained the highest levels under water stress. This suggests a strategy of active redistribution of nitrogen from older tissues to photosynthetic organs. This phenomenon was attributed to the mobilisa tion of storage proteins. The reallocation of nitrogen via the phloem helped preserve photosynthetic function and biomass synthesis (Hirel & Lea, 2001).

For phosphorus, the results obtained confirmed the strategy of preferential allocation to aerial organs in response to water stress, observed in several plant species (Plaxton & Tran, 2011).

This maintenance of foliar P content, even under water deficit, is the result of active regulation of root absorption and translocation processes via the xylem, for metabolic reactions such as oxidative phosphorylation, photosynthesis, and nucleotide metabolism (Raghothama, 1999).

The stronger reduction in root P absorption compared to leaves can be explained by the strong dependence of its transport on mobility in the soil, a process governed by water mass flow and diffusion. In particular, phosphorus, mainly present as $H_2PO_4^-$ or HPO_4^{2-} ions, is poorly mobile in soil, and its availability decreased significantly under low-humidity conditions (Hinsinger, 2001). Despite this, the phenomenon was less pronounced in Rizki, which maintained better P absorption efficiency under moderate water stress (60% FC). This suggested that Rizki was able to adapt to the water deficit by increasing the surface area of its root system and the

number of absorbent hairs, or by activating phosphate transporters (PHT) (Liao et al., 2001).

Similarly, the relative stability of phosphate levels in the leaves of Fix plants was attributed to rhizobian symbiosis, which indirectly promoted phosphate acquisition. Several studies have shown that rhizobia can stimulate root growth and modulate the expression of P transport genes via auxin production. Furthermore, nutritional synergy between P and organic nitrogen generated by symbiosis can stimulate the synthesis of proteins involved in P assimilation (McNeill AM et al., 2005). Therefore, Rizki's tolerance under symbiotic conditions (Fix) may have resulted from better integration of nutrient flows, where the availability of biological nitrogen enhances the plant's ability to mobilise and redistribute P to metabolically active tissues. Similar phenomena has been reported in other legumes subjected to drought, where N-P co-optimisation has maintained photosynthesis and delayed leaf senescence (Thilakarathna & Raizada, 2017).

For essential cations, Rizki stands out for its greater ionic plasticity and early tolerance to water stress, characterised by controlled accumulation of K^+, Ca^{2+}, Mg^{2+} , and Mn^{2+} in its aerial organs under moderate stress. It also preserves foliar nitrogen and effectively maintains symbiotic atmospheric nitrogen fixation in its nodules. This strategy suggests better regulation of ion transporters, good membrane stability, and optimised carbon allocation to nodules, allowing photosynthetic function to be maintained even under water stress (Taiz, L et al., 2018).

In contrast, Bouchra displayed low metabolic flexibility, with rapid decreases in K^+, Ca^{2+} and Fe^{2+} and marked inhibition of atmospheric nitrogen fixation activity in its nodules (-48.57% at 70% FC). This difference in behaviour between the two associations highlighted that water stress tolerance is not a monogenic trait, but rather an integrated set of physiological, nutritional, and symbiotic responses (Serraj & Sinclair, 2002). Rhizobian symbiosis, although beneficial under optimal conditions, limited host plant metabolism under moderate to severe drought stress. It reduced micronutrient nutrition (Fe, Zn^{2+}, Mn^{2+}), xylemic translocation of several cations (K^+, Mg^{2+}, Ca^{2+}), and nitrogen fixation, due to energy competition between ATP production for fixation and that required for active ion uptake (Graham & Vance, 2003). This nutritional disruption was very pronounced in Bouchra.

In general, the redistribution of nitrogen and cations to leaves is an active survival strategy that preserves photosynthetic function and biomass synthesis (Masclaux-Daubresse et al., 2006). This process, observed for N, K^+, Mg^{2+}, and Mn^{2+}, depends on protein mobilisation, phloem reallocation, and calcium signalling (Kudla et al., 2018). Its effectiveness is largely genotype-dependent.

In conclusion, Rizki has adopted a strategy of active tolerance to drought stress, characterized by a gradual and controlled accumulation of Na^+ in vegetative tissues, through effective ion management, even under symbiotic association. In contrast, Bouchra exhibited increasing ionic instability, particularly in symbiotic plants, which is probably due to its sensitivity to combined water and nitrogen deficiencies. These findings highlight that water stress tolerance results from the interaction among nutrition, ionic homeostasis, and energy status, which are determined by the genome of each variety. Consequently, selecting varieties combining efficient nitrogen fixation with ionic resilience is crucial for sustainable chickpea production in regions affected by climate change.

Analysis of the ionic status of nodules in leguminous plants, particularly chickpeas (*Cicer arietinum* L.), is a key indicator of the efficiency and effectiveness of rhizobian symbiosis. Our results clearly showed that the ionic status of nodules in essential cations (K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+} and Mn^{2+}) and toxic ions (Na^+) was affected by water stress, directly reducing biological fixation of atmospheric nitrogen and nodule performance. For the most severe stresses (60% and 50% of field capacity), nodulation processes were completely suppressed.

Nitrogen (N): The decrease in nitrogen content in Bouchra nodules resulted primarily from the inhibition of nitrogenase, which was highly sensitive to reduced cell turgidity and the accumulation of reactive oxygen species (ROS) during water stress (Serraj et al., 1999). Drought stress further restricted carbohydrate supply to nodules, thereby reducing the energy available for their metabolic activity (Rubia et al., 2025). In contrast, Rizki maintained better nitrogenase activity, likely due to more effective regulation of water homeostasis, efficient sugar translocation, and increased production of protective compounds (e.g., proline, non-reducing sugars).

Phosphorus (P): Phosphorus is essential for ATP production, respiration, and cell signalling. Its concentration decreased under drought stress, due to reduced ion mobility in the soil and decreased root transporter activity (Mahmood et al., 2025). However, both associations exhibit similar responses, suggesting that their P acquisition mechanisms (e.g., organic acid exudation, phosphatase production) are comparable. Rizki may have better P use efficiency, as observed in some stress-tolerant *Rhizobium* strains (Zahran, 1999).

Potassium (K): Potassium plays a central role in osmotic regulation, stomatal opening, and enzyme activation. The decreased in K in nodules reflected a disruption in ionic homeostasis. Rizki retained a higher K content, which explains its ability to maintain ionic gradients via specific transporters (such as voltage-sensitive K^+ channels), as described in Medicago truncatula under stress (Fedorova et al., 2020). This ability contributes to membrane stability (Sharma et al., 2013) and stress resilience.

Sodium (Na^+): Unlike other ions, Na^+ accumulated in nodules under drought stress, probably due to the breakdown of ion exclusion mechanisms. This excessive Na^+ accumulation disrupted the K^+/Na^+ balance, affecting cell function and enzyme activity (Munns & Tester, 2008). Specifically, Rizki

× *Rhizobium* strain B95 association showed higher Na^+ accumulation, which could indicate reduced efficiency in exclusion systems (such as Na^+/H^+ antiporters in the tonoplast or plasma membrane). Conversely, Bouchra × *Rhizobium* strain B95 appeared to control this accumulation better, suggesting a potential advantage in saline or semi-arid environments.

***Calcium (Ca^{2+})*:** Ca^{2+} acts as a signal in stress perception. It's gradual accumulation under water deficit, which was more pronounced in Rizki, could reflect a more intense activation of signalling pathways (e.g.Ca^2-dependent protein kinases) involved in the adaptive response (Kudla et al., 2018). However, excessive accumulation can become toxic, disrupting mitochondrial functions and nodular respiration.

***Magnesium (Mg^{2+}), Iron (Fe^{2+}), Zinc (Zn^{2+}) and Manganese (Mn^{2+})*:** These micronutrients are essential for photosynthesis (Mg, Mn), antioxidant systems (Mn-SOD, Fe-SOD), and enzymatic processes (Zn in metalloenzymes). Their decrease under water deficit related to lower transpiration and reduced movement in the xylem (White & Broadley, 2009). Rizki's slight advantage in preserving these elements may reflect more efficient chelation (e.g., phytosiderophores) or intracellular transport, as seen in some *Rhizobium leguminosarum* strains
(Reichman SM, 2007).

Meanwhile, nodule suppression by the most severe water stresses (50% and 60% FC) may have been due to:

- The limitation of flavonoid diffusion, which is essential for the induction of nod genes in rhizobia (Kunert et al., 2016; Shah & Smith, 2020; Yeremko et al., 2025) ;
- Disruption of signalling between the host plant and the *Rhizobium* strain (Fujikake et al., 2002; Yeremko et al., 2025) ;
- Reduction of carbohydrates (Kunert et al., 2016) ;
- Accumulation of reactive oxygen species (ROS) (Zhou et al., 2021) ;
- Accumulation of stress hormones (ABA), oxidative stress (Xu et al., 2021), and systemic regulation by nitrogen (Martin et al., 2023), Understanding these mechanisms is crucial for the development of legume varieties that are more resistant to drought stress, particularly in arid and semi-arid areas facing climate change. Therefore, nodulation is more sensitive to water deficit than plant growth, suggesting that resources are prioritised for vital functions at the expense of symbiosis (Todd et al., 2006; Vadez et al., 2007).

In conclusion, the two symbiotic associations reacted differently to water stress in terms of nodular ionic status. Rizki × *Rhizobium* strain B95 association showed better preservation of essential nutrients, suggesting greater stress tolerance, probably linked to more effective ion homeostasis, signalling, and antioxidant mechanisms. These results highlighted the importance of selecting *Rhizobium* strains adapted to specific environmental conditions to improve the sustainability of cropping systems in arid and semi-arid areas. This is because Rizki could induce better nodule differentiation and greater infection efficiency, as observed in *Phaseolus vulgaris* with high-performing strains (Athul et al., 2022).

***Conclusion*:** This study characterized the response of two Moroccan chickpea varieties with different behaviours, Rizki and Bouchra, subjected to different levels of water stress under two modes of nitrogen nutrition (mineral and symbiotic). Drought stress severely limited growth and nodulation, with total inhibition of the latter at 60% and 50% of field capacity. However, Rizki stood out for its tolerance, reflected in the maintenance of its biomass and photosynthetic pigments, more efficient symbiotic activity, and significant accumulation of proline and soluble sugars promoting osmotic regulation. In contrast, Bouchra showed marked sensitivity, characterised by a rapid decline in photosynthesis and a sharp decrease in nodular efficiency.

Two parameters appear to be relevant criteria for variety selection: the stability of carotenoids, which protect chlorophyll pigments, and the specific increase in Mn^{2+} in the stems, observed only in Rizki. This phenomenon suggests an ionic adaptation mechanism that maintains mineral homeostasis and potentially strengthens antioxidant defence. This is because Mn^{2+} is a key cofactor of superoxide dismutase (SOD) (Alscher et al., 2002; Broadley et al., 2007). This type of specific ionic response has rarely been documented in legumes under water stress, making it a marker of interest for selection (Ghanem M. E et al., 2020).

These results highlight the importance of incorporating morpho-physiological, biochemical, and nutritional traits into chickpea breeding programmes to identify and promote effective, drought-tolerant symbiotic associations. Thus, the rhizobian symbiosis of Rizki appears to be an agronomic asset that can be exploited in arid and semi-arid areas, where mineral nitrogen inputs remain limited or costly.

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SDGs addressed: Zero Hunger, Responsible Consumption and Production and Climate action.

***Policy referred*:** Supports FAO and INMS frameworks promoting efficient nitrogen use and reduced fertilizer dependence; aligns with UNCCD and Climate-Smart Agriculture (CSA) goals for drought resilience and soil health

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